PIXE Analysis in Different Stages of Psoriatic Skin

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Elemental distribution in psoriatic skin varies with the functional state of the keratinocytes, e.g., electrolytes influence cell metabolism and cell proliferation, and trace elements play a crucial role in a great number of enzymes.

Elemental distribution in pinpoint lesions, old plaques, and uninvolved skin of 5 psoriatic patients and 4 healthy controls was studied by means of PIXE (proton-induced x-ray emission) analysis. This technique allows the simultaneous detection of elements with an atomic number ≥14 along the epidermis and dermis in freeze-dried skin biopsies. Trace elements such as Fe, Cu, and Zn were determined down to a level of 1 ppm.

In comparison with uninvolved skin, concentrations of P and K were elevated in psoriatic epidermis. In addition, increased levels of K were correlated with the stage of the psoriatic lesion. Zinc concentrations were significantly elevated in pinpoint lesions. The Zn concentration profiles within the epidermis and upper dermis showed high correlation to the P concentration profiles. Iron levels were decreased in old psoriatic plaques, whereas Cu concentrations varied considerably. In comparison to the controls, Cl concentrations were markedly decreased in the dermis of involved and uninvolved psoriatic skin, whereas epidermal Cl levels were unaffected.

As high K levels prevent the Ca-induced differentiation of keratinocytes, high K levels may be the cause of the high cell differentiation in psoriatic skin. Elevated DNA- and RNA-polymerases might be the cause of elevated Zn levels in pinpoint lesions. *J Invest Dermatol 88:223–226, 1987*

Psoriasis vulgaris is a chronic inflammatory skin disease with hyper- and parakeratosis. Since the elemental distribution varies with the functional state of the keratinocytes, investigations have been focused on electrolytes, because of their influence on cell metabolism and cell proliferation [1], as well as on trace elements such as Fe, Cu, and Zn, because of their crucial role in a great number of enzymes [2]. The role of Zn in psoriatic lesions was first estimated by histochemical methods [3]. Later on, trace elements were determined by neutron activation analysis in mechanically separated psoriatic epidermis, which revealed increased levels of Zn [4] and Fe [5] and decreased Co concentration [6]. Recently, the elemental composition of psoriatic skin was investigated by means of x-ray microanalysis (STEM mode: scanning transmission electron microscope), revealing higher levels of P and Ca in the stratum corneum [7]. Applying the same technique, increased concentrations of Mg, K, and P were found in the involved psoriatic epidermis as a typical pattern of highly proliferative, non-neoplastic cells [8]. Further investigations on the elemental distribution in normal human and mammalian skin by x-ray microanalysis (STEM mode) have been previously described [9,10].

Microanalysis in human skin using the PIXE (proton-induced x-ray emission) method was recently reviewed by Gonsior et al [11] and Forslind et al [12]. In comparison with the STEM method, PIXE analysis achieves lower detection limits, which allows determination of elements down to levels of 1 ppm [13]. Thus, PIXE plays an important role in the quantification of trace elements such as Fe, Cu, and Zn, which cannot be determined by the STEM method [12,14]. For instance, deposits of gold in the dermis of patients with antirheumatic gold therapy were recently demonstrated [15]. Another advantage of the PIXE method is the use of cryostat sections without prior embedding procedure, so that erroneous elements can be avoided.

The present investigation was made to quantify elemental distribution, including trace elements in pinpoint lesions, old psoriatic plaques, and uninvolved psoriatic skin. This should help us to estimate the role of the different elements in the development of a psoriatic lesion.

MATERIALS AND METHODS

The study included 5 psoriatic patients, 3 males and 2 females, aged 20–48 years, with 5–21 years duration of disease. All 5 psoriatic patients presented with all types of psoriatic lesions, e.g., early pinpoint lesions and old psoriatic plaques. In all psoriatic patients, more than 30% of the skin was involved. Four healthy test persons, 2 males and 2 females, aged 26–43 years, served as controls.

Biopsies were taken from the lumbar region of the 5 psoriatic patients and the 4 controls. None of the patients was on systemic treatment. Except petrolatum, no local treatment was applied for 2 weeks prior to taking the biopsies. After anesthesia with 1 ml Xylocain 1% s.c., biopsies were obtained from an early psoriatic lesion with a maximum diameter of 3 mm (pinpoint lesion), an old psoriatic plaque, and uninvolved psoriatic skin, approximately 5 cm from a psoriatic lesion. The biopsies were frozen and stored in liquid nitrogen at −196°C. Cryosections of 10 μm

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Abbreviations:

PIXE: proton-induced x-ray emission

STEM: scanning transmission electron microscope

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thickness were cut perpendicular to the skin surface at a temperature of −20°C. The sections were fixed to thin (<0.5 μm) Formvar foils without any supplement, smoothed by short-term thawing (some seconds at room temperature), and then completely dried at a temperature of −20°C. This resulted in easier sample handling. Moreover, the absence of a covering foil reduced the energy loss of the projectiles and resulted in a lower x-ray absorption.

In order to investigate the ion movement in the thawed sections, these samples were compared to sections completely freeze-dried between 2 Formvar foils as well as to completely thawed and dried cryosections at +20°C. The comparison was accomplished by analyzing neighboring parallel sections treated in the different ways as described. In all 3 cases, the elemental distributions did not markedly differ in regions larger than the cell dimension. Differences may be founded on the fact that even neighboring parallel sections are not completely identical. The drying process seems to be much faster than the diffusion of ions through the cell membranes. In addition, similar mass distribution profiles for most of the elements were found by other authors [12] using completely freeze-dried samples.

The PIXE method in combination with the Bochum proton microprobe [13] was used for the determination of the element distributions. This method is similar to the electron microprobe analysis and uses protons instead of electrons as projectiles. The advantage of the PIXE method is a lower detection limit down to 1 ppm. The disadvantage of the poorer lateral resolution is not of great importance in the case of samples of some micrometers in thickness, because the electron beam would be scattered at the sample atoms to ~1 μm diameter while the scattering of the proton beam is negligible. The Bochum proton microprobe is equipped with a magnetic scanner and a PDP 11/44/CAMAC data handling system [14].

The analyses described in this paper were accomplished using a lateral resolution of ~ 5 × 5 μm^2, a beam current of 1 nA, and a proton energy of 3 MeV. In each of the samples the distribution of the elements P, S, Cl, K, Fe, Cu, and Zn as well as the sample mass per unit area were determined quantitatively taking a line-scan perpendicular to the skin surface. Calibration was done by measuring the elastic scattering of the protons at the target nuclei. The matrix mass per unit area was determined by evaluating the secondary electron bremsstrahlung background in 2 energy ranges: (1) 2 channels in the well-defined minimum between the $Cl\bar{K}$- and the KK_α-peaks, and (2) the background within the range from the minimum between the $Cl\bar{K}$- and the KK_α-peak to the minimum between the KK_β/CaK_α- and the CaK_β-peaks. Normally both ranges are free from any pileup effect. A Kapton foil (830 $\mu g/cm^2$) was used for calibration. After the measurements, microphotos of the irradiated samples were taken. The dermal-epidermal junction is clearly visible in most cases without any staining.

The significance levels of differences between samples from psoriatic patients and samples from the healthy control group were calculated using Student's *t*-test. For the comparison of involved and uninvolved skin a paired *t*-test was used.

RESULTS

Elemental distribution in freeze-dried skin sections of 5 psoriatic patients and 4 controls was measured by means of the PIXE method. The irradiated areas could be microscopically visualized as a dark line (Fig 1). In order to localize the stratum corneum and the basal layer, a picture was taken from each skin section measured. Concentration profiles could be obtained in the epidermis and upper dermis of the following elements: P, S, Cl, K, Fe, Cu, and Zn. Calcium concentrations could not be exactly determined, for the calcium K_α lines interfered with the potassium K_β lines, so that high concentrations of K rendered the detection of Ca levels rather inaccurate. Thus, measured Ca concentrations, ranging from 100–400 ppm, varied considerably and did not show any significant differences.

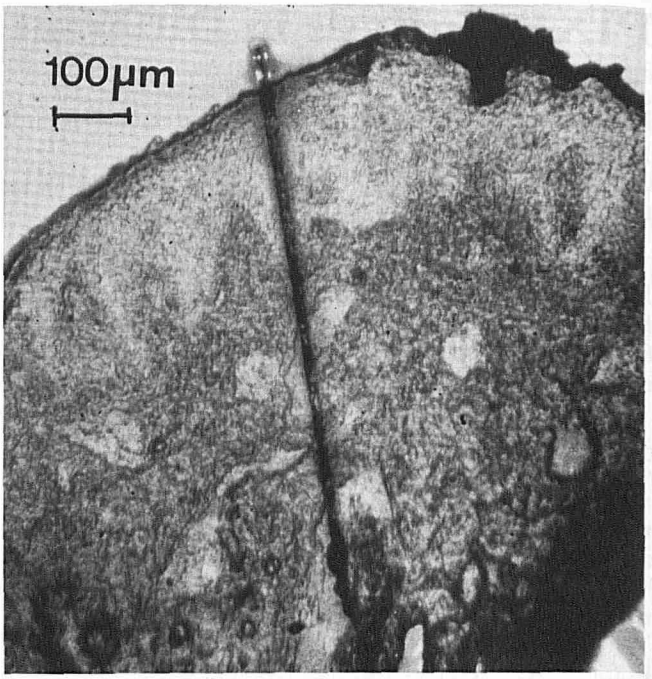

Figure 1. Micrograph of a dry cryosection of psoriatic skin (pinpoint lesion) after measurement with the PIXE system. Irradiated areas (proton beam) are visualized as a dark line.

Each concentration profile was plotted in a diagram (e.g., Fig 2); mean values in the epidermis and dermis were calculated. Our results are summarized in Table I. Compared to the controls, we found significant differences in dermal concentrations of Cl, which were reduced in involved and uninvolved psoriatic skin. Concerning the epidermis, Cl levels were unaffected, showing a linear increase from the stratum corneum down to the basal layer (Fig 2). No other elements showed any marked differences in dermal concentrations. Within the epidermis, all striking differences were found between involved and uninvolved psoriatic skin. The involved psoriatic epidermis showed increased concentrations of K, P, and Zn, as well as decreased levels of Fe. We could demonstrate a linear correlation between the elevated K levels and the stage of the psoriatic lesion (Fig 2). Compared to the controls, K concentration seemed to be lower in uninvolved psoriatic skin, but the difference was statistically not significant. We found markedly elevated Zn levels in pinpoint lesions, whereas in old psoriatic plaques Zn concentrations were unaffected. The concentration profiles of Zn were remarkably related to those of P in all psoriatic skin samples (e.g., Fig 2). The levels of S in psoriatic patients resembled those in the controls, sometimes showing slight concentration maxima in the upper epidermis. The Fe levels were significantly decreased in old psoriatic plaques, whereas they varied considerably in the controls. The concentrations of Cu were close to the detection limit, showing varying concentration profiles. In addition, the serum levels of Na, K, Ca, and Cl did not show any significant differences between psoriatic patients and controls.

DISCUSSION

The PIXE method is a microanalytical technique for the simultaneous determination of elements with an atomic number ≥14 as previously described [11]. Compared with the electron beam x-ray microanalysis (STEM method), the PIXE method has a higher sensitivity. Thus, trace elements such as Fe, Cu, and Zn can be quantified down to a concentration of 1 ppm [14]. In the present investigation, the PIXE analysis was applied for the determination of the elemental distribution in different stages of psoriatic skin. Summarizing the results in comparison to unin-

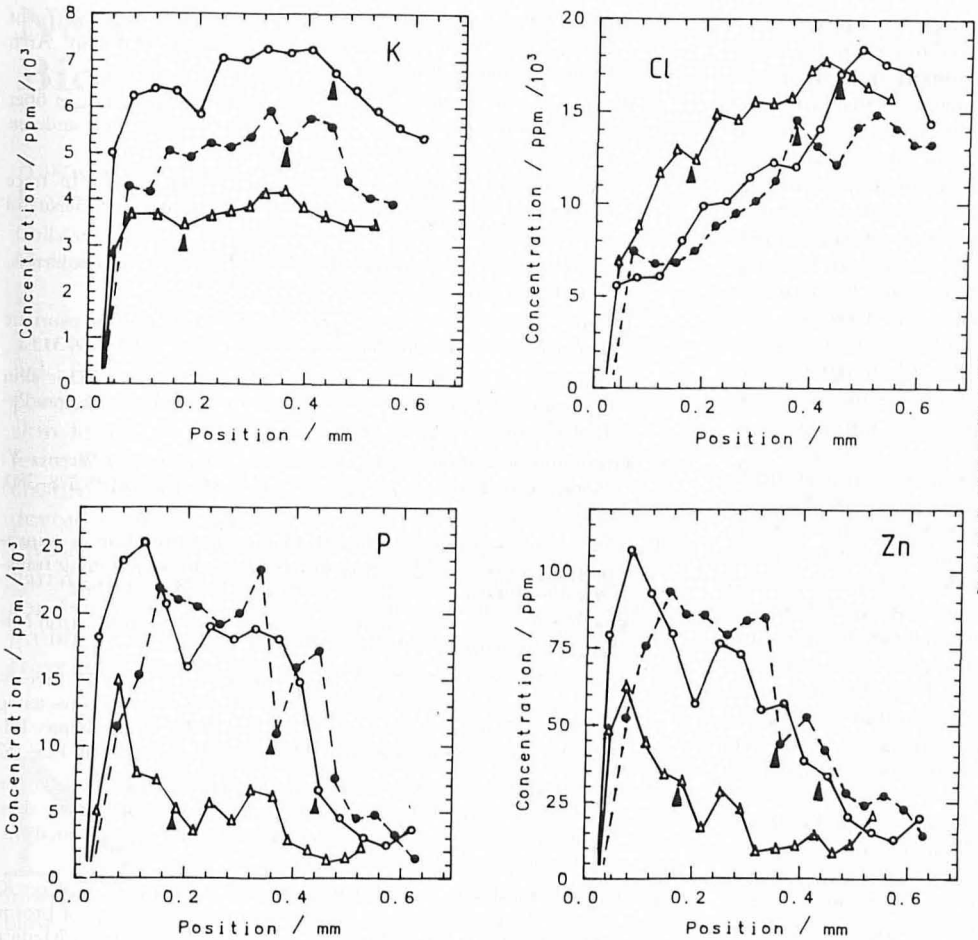

Figure 2. Elemental concentrations in cryosections of 1 representative patient: potassium (*K*), chlorine (*Cl*), phosphorus (*P*), and zinc (*Zn*). The stratum corneum starts approximately at position 0 mm, the black *arrowheads* indicate the dermal-epidermal junction. *Open circles* = old psoriatic plaque, *closed circles* = pinpoint lesion, *open triangles* = uninvolved psoriatic skin. All concentrations are given in μg/g (ppm) dry weight.

volved psoriatic skin, we found increased levels of K and P in psoriatic epidermis, elevated Zn concentrations in pinpoint lesions, and decreased Fe levels in old psoriatic plaques. Compared to the controls, the Cl concentrations were significantly decreased in involved and uninvolved psoriatic dermis. In general, most differences regarding the elemental distribution were found between involved and uninvolved psoriatic epidermis, and not between patients with psoriasis and controls. This may be due to the relatively high interindividual variability observed in psoriatic patients and controls. Any localization factors could be excluded as all measured biopsies were taken from the lumbar region. Exact determination of Ca was prevented by high K concentrations.

In previous investigations, we used a drop of water to attach the skin sections to the plastic foils [14,15]. This procedure led to artifacts, as the diffusible ions, such as K, were resolved in unknown amounts (70–90%), so that low Ca levels could be detected more exactly. To prevent those artifacts, the skin sections in the present investigation were attached to the plastic foils without using a solvent. Thus, higher K concentrations occurred. Detection of Ca might be improved by applying lower proton energies and a thinner detection window in the PIXE system.

It has been suggested that elevated K concentrations in correspondence with the stage of the psoriatic lesion may be the result of hyper- and parakeratosis [16]. On the other hand, K can cause changes with regard to the proliferation and differentiation of cultured epidermal cells. It has been demonstrated that low K levels prevent the Ca-induced differentiation, leading to highly proliferative and little differentiated keratinocytes [1]. Thus, the marked differentiation of the highly proliferating psoriatic keratinocytes could be caused by elevated K levels. This theory is supported by investigations of Nagy [17], who found low or unaffected K levels and high Na levels in undifferentiated cancer cells, leading to a high Na/K ratio as a typical pattern for highly proliferating neoplastic cells. Recently, in the psoriatic epidermis, a slightly decreased Na/K ratio was found as a typical pattern for rapidly dividing non-neoplastic cells [8]. Summarizing these results, we assume that high K levels can promote the differentiation of psoriatic keratinocytes.

Table I. Elemental Concentration of Normal and Psoriatic Skin

	P	S	Cl	K	Fe	Cu	Zn	Cl (Dermis)
Control	12,500 ± 4,300	11,400 ± 900	10,200 ± 3,900	8,900 ± 2,200	240 ± 225	8.3 ± 0.6	77 ± 22	20,400 ± 2,000
Uninvolved psoriatic skin	12,000 ± 3,700	10,700 ± 2,100	9,300 ± 2,500	6,800 ± 1,900	98 ± 54	12.7 ± 6.5	64 ± 20	14,400 ± 3,300[c]
Pinpoint lesion	18,400 ± 2,700[b]	11,500 ± 2,100	8,600 ± 2,800	8,800 ± 4,000[a]	55 ± 23	9.9 ± 4.5	85 ± 11[b]	15,100 ± 1,900[d]
Old psoriatic plaque	16,600 ± 1,900[b]	11,000 ± 1,800	9,300 ± 2,500	11,000 ± 3,800[b]	59 ± 15[b]	14.9 ± 8.3	71 ± 7	14,100 ± 2,500[d]

Values are given for elemental concentration in the epidermis and in 1 case in the dermis (Cl), as means ± SEM in 4 controls and 5 psoriatic patients. Concentrations are given in μg/g (ppm) dry weight.

[a,b]Significantly different from uninvolved psoriatic skin (*a*, $p < 0.05$; *b*, $p < 0.01$).

[c,d]Significantly different from control (*c*, $p < 0.02$; *d*, $p < 0.01$).

The markedly decreased Cl concentrations we found in involved and uninvolved psoriatic dermis have not been described yet. Since Xylocain (10 mg lidocain hydrochlorate and 6 mg NaCl in 1 ml H_2O) was used as local anesthesia, an effect of these substances might be discussed. However, Xylocain was applied in the same way in both groups, patients with psoriasis and controls, so that these artifacts may be excluded. Since decreased Cl levels were found in involved and uninvolved psoriatic dermis, a quicker diffusion of Xylocain in the inflammatory skin cannot be the reason for these results. It is difficult, however, to interpret all dermal data because it was not possible to measure the complete concentration profiles along the dermis. Thus, the decreased psoriatic Cl concentrations should be valid only in the case of an approximately constant Cl profile along the whole dermis. On the other hand, the dermal concentrations of all other elements varied considerably, and further investigation is required to illuminate this part of the psoriatic skin.

The increased P levels in lesional psoriatic epidermis could be explained by higher contents of nucleic acids [17] or higher concentrations of phospholipids [18]. The latter possibility seems to be more likely in view of the remarkable similarity of the Zn and P concentration profiles in psoriatic skin. As Zn levels were significantly elevated in pinpoint lesions, we assume that DNA- and RNA-polymerases dependant on Zn were elevated, increasing the metabolic turnover and the proliferation rate of the keratinocytes in the early stage of a psoriatic lesion [19]. Alkaline phosphatase is another enzyme dependent on Zn found to be increased in psoriatic skin [20]. Since Zn plays a crucial role in more than 70 enzymes [21], however, speculations on high Zn levels in psoriatic epidermis will continue.

In contrast to former investigations showing elevated Fe levels in psoriatic skin [5], we found decreased Fe levels in old psoriatic plaques. Those authors discussed a marked loss of Fe by epidermal desquamation in psoriatic patients. Our results do not support this theory. Since neutron activation analysis was applied in mechanically separated epidermis, the difference is perhaps caused by applying another method.

Our results are in good agreement with the data obtained by Grundin et al [8], who investigated psoriatic epidermis using the electron probe x-ray microanalysis. They additionally found elevated Mg levels and unaffected concentrations of Na, but, as they were limited by their detection method, they were not able to determine trace element concentrations.

The PIXE method used in the present investigation allows the determination of the elemental distribution including trace elements such as Fe, Cu and Zn in psoriatic patients and controls. In psoriatic skin, we could demonstrate marked changes regarding the concentrations of epidermal K, P, Fe, and Zn and decreased levels of dermal Cl. Thus, the PIXE method constitutes a valuable quantitative technique in addition to electron probe x-ray analysis and morphologic methods. For better understanding of the measured results, however, further investigations applying the PIXE technique in other skin diseases are required.

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